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Chemical Signal-to-Noise Detection by Spiny Lobsters

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Abstract. The smallest difference in concentration detected between a chemical stimulus and background is called the threshold of "just noticeable difference" (*jnd*). Measurements of *jnd* thresholds have been made extensively in psychophysical research on olfactory and taste perception by terrestrial mammals, but not on chemoreception by marine organisms. Marine organisms live in a persistently noisy chemical environment, because stimulatory compounds are often components of the background in seawater. Measurements of *jnd* thresholds, therefore, should be especially appropriate in the marine environment and were the focus of this study. Laboratory assays were used in measuring the ability of spiny lobsters, *Panulirus interruptus*, to detect a glycine stimulus against a background concentration of glycine. Glycine was chosen as stimulant because it is a major component of dissolved organic matter (DOM) in seawater, is abundant in prey tissues, and is excitatory to the appetitive feeding phase of *P. interruptus*. Chemical determinations of glycine in seawater were made by reverse-phase, high-performance liquid chromatography. The *jnd* threshold that was estimated in this study for glycine detection by lobsters was about 2–8% above the background concentration of glycine in seawater. This threshold is slightly lower than ones demonstrated for odorant detection by humans and other terrestrial animals. Consequently, the olfactory sense of lobsters appears to be well constructed to detect subtle changes between concentrations of stimulus and background, a facility that may be important in the ecology of this animal.

Introduction

Chemical stimuli are important factors controlling the behavior of aquatic organisms. Chemical cues often mediate predator-prey interactions (Peckarsky, 1980; Croll, 1983; Zimmer-Faust, 1989; Sih and Moore, 1990), competition (Caldwell, 1982; Sammarco *et al.*, 1983; La Barre *et al.*, 1986), courtship and mating (Gleeson *et al.*, 1984; Miller, 1989), gregariousness and sociality (Zimmer-Faust and Spanier, 1987; Jensen, 1989), and habitat selection (Hadfield and Scheuer, 1985; Raimondi, 1988; Sweatman, 1988; Morse, 1990). Several properties of chemical cues, including molecular structure, concentration, and distribution in time and space, contribute to the stimulation of a behavioral response.

The ability to detect concentration differences is especially important, because instantaneous or time-averaged gradients in chemical concentration may provide animals with valuable information about their distance and direction from the odor source (Moore and Atema, 1988; Zimmer-Faust *et al.*, 1988). The source may be food or a mate, and chemical concentration might also provide information about the quantity or quality of the resource. Because many stimulatory molecules occur naturally as dissolved organic matter in seawater, marine organisms face the problem of resolving chemical signals from environmental background, or chemical "noise." In particular, free amino acids, nucleotides, sugars, and organic acids function as signals of food (see reviews of Carr, 1988; Laverack, 1988), but they also occur abundantly in seawater. The effects of chemical stimulus concentration have been considered in many studies on marine animal behavior (Ache, 1982; Carr, 1988; Zimmer-Faust, 1989), but in no investigation have response thresholds to applied stimuli been measured relative to background concentrations. Because many chemoreceptors function as detectors of relative changes in concentration, threshold determinations may be products both of animal sensitivity to tested compounds, and to background chemical levels in seawater.

The threshold of "just noticeable difference" (*jnd*) is the smallest difference detected between the concentration

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of the stimulus and that of the background. The *jnd* threshold has been used extensively in psychophysical research on olfactory and taste perception by vertebrates, especially humans (*sensu* McBurney *et al.*, 1967), but has not been applied to investigations of chemoreceptive behavior in marine organisms. Yet measurements of *jnd* thresholds should be especially appropriate to marine organisms, which live in a persistently noisy chemical environment. In fact, threshold determinations of *jnd* should be essential to understanding the environmental constraints imposed on chemical detection. For these reasons, during the present study, analytical procedures useful for measuring *jnd* thresholds of chemical detection by marine organisms were developed. The procedures were then applied in estimating the threshold of *jnd* for chemical detection by the spiny lobster, *Panulirus interruptus*.

Materials and Methods

Collection and maintenance of animals

Lobsters were captured by SCUBA divers and were brought immediately to the laboratory where small groups of individuals were placed in large, outdoor circular tanks (1.5 m diameter × 0.6 m height) having excess shelters (see Zimmer-Faust and Spanier, 1987). A continuous seawater flow (5 μm filtered) of 8 l/min maintained the aeration and temperature (15–16°C) of each holding tank. Incoming animals were tattooed on their ventral thoracic sternites, permitting individual recognition (Kuris, 1971), while gender and reproductive status were noted. Only hard-shelled animals, 60–69 mm carapace length, were used in the experiments. All animals were fed *ad libitum* on live mussels (*Mytilus californianus* and *M. edulis*), sea urchins (*Strongylocentrotus purpuratus*), and polychaete worms (*Phragmatopoma californica*), but were deprived of food for 24 h before testing.

Experimental chambers

Individual lobsters were tested for responses to chemical solutions presented in rectangular chambers, 30 × 30 × 15 cm, constructed so as to allow careful control of test stimulus flow characteristics. Opaque blinds around each chamber permitted us to observe the test animals without disturbing them. A diffuse red illumination, completely confined by the blinds, was provided, and the surrounding laboratory was maintained in darkness. Seawater (single-pass; 5 μm filtered) entered each chamber by a delivery system held under constant hydrostatic pressure. Polyethylene tubing carried a primary seawater flow (936 ml/min) from a head-tank to an adaptor positioned above each chamber; from the adaptor, the seawater was delivered to the center of the chamber, 0.5 cm below the water surface. A valve in each delivery line enabled fine adjustments of water flow. A secondary flow (122 ml/min) was carried by polyethylene tubing from a head-tank to a stimulus reservoir before it joined the primary flow at the adaptor. Test stimulant solution was introduced (10 ml/7 s) when a three-way valve that connected the stimulus reservoir to the secondary flow system was opened. A fitting in each adaptor eliminated back-flow, ensuring that seawater and test stimulants would pass from the secondary to primary flow before entering a chamber.

Choice of glycine as test stimulus

Glycine was used as test stimulant because it is excitatory to the appetitive phase of feeding by *P. interruptus* (Zimmer-Faust *et al.*, 1984; Zimmer-Faust, 1987). It is also abundant in the tissues of invertebrate prey consumed by lobsters (*e.g.*, Bowlus and Somero, 1979; Zurburg and DeZwaan, 1981; Yancey *et al.*, 1982) and is released by some prey as a metabolite (Zimmer-Faust, unpub. data). Because glycine is one of three most abundant amino acids in coastal seawater (Clark *et al.*, 1972; Dawson and Pritchard, 1978; Garrasi *et al.*, 1982; Mopper and Lindroth, 1982; Fuhrman and Bell, 1985), it is an ecologically relevant stimulus molecule that lobsters must naturally detect from background noise.

Experimental procedures

Lobsters were placed in experimental chambers 45 to 60 min before testing, and they usually acclimated within 30 min. Observations of behavior were initiated 1 min before the introduction of a test or control solution and were continued for 3 min afterwards; the observer was unaware of the solution being tested. In each trial, a lobster was presented with 10 ml of either 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 5×10^{-8}, or 10^{-8} *M* glycine added to the seawater as described above. Each concentration of glycine was tested 20 times, each time on a different animal. In 40 trials, lobsters were presented only with seawater (control). Each test and control solution was prepared fresh immediately prior to assay. Individual lobsters were tested only once in 48 h, for a maximum of three times during an 8-day period. Chemical solutions and their order of presentation were selected with a random-numbers table, except that identical solutions were never repetitively tested on the same animal.

Preparation of chemical solutions

Special care was taken in preparing test solutions. First, stocks were made by dissolving glycine in artificial seawater prepared with HPLC-grade DI water and analytical grade salts according to the Marine Biological Laboratory formula (Cavanaugh, 1975). The concentrations of the stock solutions were 100-times higher than those of the

test solutions. Stocks were divided in 1 ml aliquots and frozen at −20°C until used. Test solutions were made by adding 100 μl of an appropriate stock to 9.9 ml seawater; control solutions were made by adding 100 μl of artificial seawater to 9.9 ml seawater. In fact, each solution was made with seawater drawn from the same experimental chamber that was to be used in the assay. Test concentrations, measured by high-performance liquid chromatography (HPLC) (n = 14 trials), were very accurate and deviated only 2–13% from values estimated volumetrically. Glycine concentrations that were maintained in chamber seawater did not measurably change during the 3–5 min required to prepare a solution (see Results). Consequently, the glycine added with each test solution was an accurate representation of the stimulus introduced above seawater background.

Behavioral assays

Antennule flicking, antennule wiping, leg probing, and mouthpart labiating were all used as behavioral assays. Both electrophysiological and behavioral investigations have shown that flicking is associated with the detection of chemical stimuli (Snow, 1973; Pearson and Olla, 1977; Price and Ache, 1977; Pearson *et al.*, 1979; Schmitt and Ache, 1979; Rebach *et al.*, 1990). Antennule wiping against the mouthparts is believed to function either in cleaning or resetting those antennule chemoreceptors that are important to feeding (Snow, 1973; Fuzessery and Childress, 1975). While leg probing is used in contacting food and passing it to the mouth (Derby and Atema, 1982; Zimmer-Faust and Case, 1982), mouthpart labiating in preparation for eating is carried out with the endopodites on the third maxillipeds. The following responses are defined: (1) "glycine detection" is an increase in the rate of flicking, over 15 s or longer, within the 3-min test period; whereas (2) "glycine-mediated appetitive feeding" is the co-occurrence of flicking, wiping, leg probing, and mouthpart labiating, all within the 3-min test period. Thus combined, the four acts constitute the initial phases of feeding (Zimmer-Faust *et al.*, 1984), and they differ markedly from the incipient phases of other behaviors, such as socializing and predator avoidance (Zimmer-Faust *et al.*, 1985; Zimmer-Faust and Spanier, 1987).

Chemical determinations

Concentrations of glycine, ammonium, and 13 other free-amino acids in the seawater sampled from test solutions and experimental chambers were determined. Each aliquot (1 ml) was collected with a polypropylene syringe and teflon tubing immediately prior to an experimental test. Samples were filtered gently (<10 psig) to 0.22 μm, then placed in cryogenic vials, put on liquid nitrogen, and stored at −76°C. All labwares and membrane filters were washed with 10% HCl (Baker Altrex grade), rinsed with HPLC-grade DI water, and then rinsed again with sample seawater before use. Chemical determinations were made by reverse-phase HPLC.

Chemical analytical procedures followed those described previously by Jones *et al.* (1981) and Manahan *et al.* (1983). Samples were reacted with ortho-phthaldialdehyde (OPA) in the presence of mercaptans to form fluorescent products. Sub-samples (100 μl) of OPA-derivatives were withdrawn and separated on an Altex ultrasphere ODS column (150 × 4.6 mm; particle size = 5 μm; length 15 cm). A gradient was created between two sodium acetate/methanol buffers (solvent A; 400 ml sodium acetate, 95 ml methanol; solvent B: 100 ml sodium acetate, 400 ml methanol) (see Jones *et al.*, 1981), held at pH 6.8 to improve separation of phenylalanine and ammonium. The eluant buffers were controlled by a Gilson (Model 42) gradient analytical system with dual pumps (Model 302) interfaced with a PC-based programming module (Gilson Model 704). Peaks in the eluant stream were monitored with a Gilson (Model 121) fluorometer and identified by elution time, relative to known standards. Reagents and solvents all were HPLC grade, degassed prior to use.

Determination of the ratiometric dilution associated with introduction of a test stimulus solution

The dilution associated with stimulus delivery was determined for a fluorescent dye (sodium fluorescein) introduced in place of the test or control solutions (n = 24 trials). Fluorescence was monitored with a fast, multichannel fluorometer sampling continuously at 10 ms intervals over 3-min trials (Zimmer-Faust *et al.*, 1988). The fluorometer was equipped with an optical fiber probe (500 μm diameter) placed about 1 cm below the point at which the stimulus entered the test chambers. Optical fibers, as light collecting devices, have a distinct advantage over other fluorometric and spectrophotometric methods; *i.e.*, because flow cells and pumping are eliminated, water flow is not disrupted. The placement of the probe ensured that the dye and seawater would be sampled as they entered the chambers and before contacting either the olfactory (antennules) or taste (mouthparts, leg tips) organs of *P. interruptus*. Because the dilution of the fluorescein dye was estimated from values determined for peak fluorescence, subsequent calculations of stimulus concentrations were conservative and probably over-estimated slightly those concentrations actually contacting the sensory appendages.

The dilution of fluorescein dye measured in each trial, then averaged over all trials, was 0.056 (±0.11 SD) times the initial concentration introduced. This value is only slightly lower than 0.074, which is the dilution, calculated

volumetrically, from the point of stimulus (and dye) input to the point of entry into the experimental chamber, assuming uniform mixing during transport and delivery. Consequently, volumetric measurements, which are more easily obtained, also accurately estimate stimulus dilution.

Results

Chemical determinations

The glycine concentrations in the seawater of experimental chambers were sampled prior to 40 selected trials. Each of these trials was chosen at random from among all of those performed, except that determinations were distributed equally among days. The sources and magnitudes of within-day variation in glycine are difficult to assess. Duplicate seawater samples were occasionally taken at the same time from single chambers. The average range of glycine concentrations determined from the duplicate analyses is the mean ± 11%. While this range seems rather large, it is typical of those found by other investigators that have previously measured free amino acids in seawater (*e.g.*, Fuhrman and Bell, 1985). Sixty-five percent of the variance in glycine concentration is explained by differences occurring between days. The between-day variation does not differ significantly from the variation measured within each day (One-way ANOVA: d.f. = 7/32, $F = 1.93$, $P > 0.10$); thus the data justifiably can be pooled. Means and variances are given in Table I for concentrations of glycine, ammonium, and 13 other free amino acids in the seawater of experimental chambers.

Glycine also was measured for seawater drawn each day from a pair of experimental chambers randomly chosen, one tank holding a lobster and the other not. Mean concentrations in the seawater of paired tanks were found to be nearly equal before, and again 60 min after placing lobsters (Table II). These data suggest that the glycine concentrations in seawater were stable over intervals that were longer than the duration of the behavioral bioassay. Nevertheless, small, instantaneous fluctuations in glycine might well have occurred during trial intervals. The effect would have been to limit the detection of the glycine stimulus by the lobsters and to increase the threshold of *jnd* proportional to the magnitudes of the fluctuations (see arguments of Cain, 1977a). Because a very low *jnd* threshold was determined (see next section), it is unlikely that glycine fluctuated much, if at all, during the trials.

Ammonium in the seawater of experimental chambers increased significantly when lobsters were being held (Table II, and Student's *t*-test: $t = 3.96$, d.f. = 14, $P < 0.01$). Similarly, concentrations of alanine, aspartic acid, glutamic acid, histidine, and lysine all increased, but to a lesser extent. Sixty minutes after lobsters were placed in the seawater, the mean total concentration of free amino acids was 556 (±131 n*M* SD), as compared to 497 (±110 n*M* SD) before. This difference is not significant (*t*-test: $t = 0.98$, d.f. = 14, $P > 0.20$). Similarly, the mean total concentration of free amino acids in seawater without lobsters was 469 (±106 n*M* SD) after 60 min, as compared to 478 (±91 n*M* SD) before. This difference also is not significant ($t = 0.18$, d.f. = 14, $P > 0.50$).

Table I

Concentrations (nM) of dissolved glycine, ammonium, and 13 other free amino acids in the seawater of experimental chambers, immediately before stimulus introduction

Compound	Concentration
alanine	72.6 ± 36.5
ammonium	2512. ± 468.
arginine	18.2 ± 15.7
aspartic acid	81.0 ± 31.2
glutamic acid	49.4 ± 21.0
glycine	153.1 ± 52.2
histidine	17.9 ± 10.1
isoleucine	14.4 ± 6.5
leucine	18.3 ± 4.5
lysine	37.4 ± 12.6
methionine	3.9 ± 2.0
phenylalanine	11.3 ± 2.7
serine	20.5 ± 7.4
tyrosine	6.4 ± 3.5
valine	20.7 ± 11.3

Values are means (±1 standard deviation) determined for samples drawn before 40 randomly chosen trials.

Psychophysical research has shown that animals respond to changes in chemical stimulus concentrations. In fact, it is the *proportional* change in concentration, called the Weber fraction (W_f), that animals detect. The Weber fraction is the difference in concentration measured before (C_b) and after (C_a) the introduction of a stimulus, divided by the concentration before:

$$W_f = (C_a - C_b)/C_b \quad (1)$$

In this study with lobsters, the peak difference in glycine, $C_a - C_b$, was accurately estimated by multiplying the concentration (C_s) of stimulus added to the test solution by the ratiometric dilution (x) associated with stimulus delivery. The Weber fraction is then:

$$W_f = xC_s/C_c \quad (2)$$

where C_c is the concentration of glycine in the seawater of the experimental chamber immediately before stimulus entry. C_s can be treated as a constant, because it varied insignificantly (2–13%) from trial to trial for a given glycine addition (see Materials and Methods). Alternatively, both x and C_c varied significantly from trial to trial, and this associated variance can be used to derive the precision with which W_f is estimated. The distribution of W_f is sto-

Table II

Concentrations (nM) of dissolved glycine, ammonium, and 13 other free amino acids in the seawater of experimental chambers with lobsters present or absent

	Lobsters present		Lobsters absent	
Compound	Time = 0	Time = 60	Time = 0	Time = 60
alanine	72.9 ± 46.7	119.1 ± 125.9	57.3 ± 35.6	59.8 ± 38.1
ammonium	1416. ± 499.	2464. ± 349.	1386. ± 422.	1283. ± 368.
arginine	15.2 ± 8.1	14.8 ± 5.2	17.4 ± 11.7	18.7 ± 15.7
aspartic acid	74.0 ± 19.7	88.0 ± 28.8	77.7 ± 21.8	71.7 ± 41.1
glutamic acid	47.4 ± 14.0	57.5 ± 21.1	36.2 ± 17.3	35.6 ± 14.7
glycine	151.1 ± 40.4	138.7 ± 52.6	155.3 ± 23.0	150.8 ± 50.3
histidine	16.8 ± 2.9	18.4 ± 8.0	15.3 ± 8.1	14.0 ± 8.5
isoleucine	11.6 ± 1.7	11.3 ± 2.8	11.7 ± 2.1	12.0 ± 1.5
leucine	16.4 ± 2.9	16.3 ± 6.0	16.5 ± 4.6	16.6 ± 3.5
lysine	32.8 ± 10.1	35.3 ± 9.8	36.0 ± 8.2	34.7 ± 12.6
methionine	3.1 ± 2.3	3.4 ± 1.3	3.3 ± 0.8	3.3 ± 1.3
phenylalanine	10.7 ± 3.1	9.6 ± 2.3	10.5 ± 1.2	10.4 ± 2.5
serine	17.3 ± 3.4	16.4 ± 4.5	19.9 ± 6.3	20.5 ± 7.4
tyrosine	6.4 ± 1.6	5.8 ± 2.4	7.0 ± 2.1	7.7 ± 3.5
valine	21.2 ± 4.0	22.0 ± 8.5	14.6 ± 3.7	14.2 ± 2.5

Samples in each treatment were drawn from seawater of eight experimental chambers before (time = 0), and again after a 60 min holding period. Values are means (±1 standard deviation).

chastic and cauchy, because W_f is a quotient of two stochastic variables, x and C_c (see equation 2). Mean and variance of a cauchy distribution are theoretically undefined, but can be approximated (Hoel *et al.*, 1971). The steps used in this approximation are as follows. (1) Log transform all values of x (*i.e.*, $x_1, x_2, x_3 \ldots x_{24}$), C_c (*i.e.*, $C_{c1}, C_{c2}, C_{c3} \ldots C_{c40}$) and C_s (10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 5×10^{-8} and 10^{-8} *M*). (2) Apply equation 2, repetitively, using all combinations of log transformed values, x and C_c, in deriving the distribution of W_f for each constant, C_s [note that each distribution of W_f had 960 values]. (3) Finally, calculate the mean and variance of W_f for each distribution, then back transform by taking the antilogs. Because the distributions of x and C_c were always the same and only C_s (a constant) changed between derivations, the coefficients of variation ($SD/\bar{x}$) were identical for each distribution of W_f. The coefficient of variation was 0.5-times the mean, providing some indication of the precision with which W_f was measured.

Table III

Detection and appetitive feeding by lobsters to applied glycine stimuli and seawater (control)

		Proportion responding[a]		
C_s	W_f	Detection	Feeding	Number tested
1×10^{-2}	$3.65\ (\pm 1.79) \times 10^{3}$	1.00***	0.75***	20
1×10^{-3}	$3.65\ (\pm 1.79) \times 10^{2}$	1.00***	0.60***	20
1×10^{-4}	$3.65\ (\pm 1.79) \times 10^{1}$	1.00***	0.55***	20
1×10^{-5}	$3.65\ (\pm 1.79) \times 10^{0}$	1.00***	0.20*	20
1×10^{-6}	$3.65\ (\pm 1.79) \times 10^{-1}$	0.95***	0.00	20
1×10^{-7}	$3.65\ (\pm 1.79) \times 10^{-2}$	0.80**	0.00	20
5×10^{-8}	$1.82\ (\pm 0.90) \times 10^{-2}$	0.70*	0.00	20
1×10^{-8}	$3.65\ (\pm 1.79) \times 10^{-2}$	0.55	0.00	20
(seawater control)				
0	0	0.38	0.00	40

[a] The proportion tested and responding to stimulus is significantly different from the proportion tested and responding to seawater (control). (*G*-test with Yates' correction for continuity: d.f. = 1, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$).

C_s is the molarity of glycine added to the stimulus solution, while W_f is the Weber fraction. The lowest W_f detected is the threshold of *jnd* Values of W_f are expressed as means ±1 standard deviation).

Lobster responses to applied glycine stimuli

An application of a glycine stimulus was considered excitatory if the proportion of lobsters tested and responding was significantly greater than the proportion tested and responding to seawater (control). On this criterion, the threshold of *jnd* determined for glycine detection by lobsters was 1.8% (±0.9% SD) above background (Table III). Lobsters were less sensitive in responding to glycine as a feeding cue. Behavioral induction required an applied dose, at least 3.65-times above background (Table III).

Although the threshold of *jnd* for glycine detection seems quite low, several lines of evidence suggest that the estimate is accurate. First, great care was taken in the measurement of glycine concentrations and ratiometric dilution. Ratiometric dilution was calculated from values

of *peak* fluorescence recorded in trials in which dye was released. Consequently, dilution associated with stimulus delivery was the *minimum* observed. Second, the threshold of *jnd* is little affected by the method of estimating ratiometric dilution, whether fluorometric or volumetric. Substituting a value calculated volumetrically, 0.074, produces a threshold of *jnd* that is 2.4% above background. Third, values calculated for W_f are very robust. It follows from equation 2 that W_f increases as x increases, as C_c decreases, and as x increases, while C_c decreases. Substituting a value of x two standard deviations above the mean, while also substituting a value of C_c two standard deviations below the mean, yields a threshold of *jnd* that is 8.0% above background. Finally, the duration of increased antennule flicking was shortened in response to the lowest stimulus concentrations tested. For example, lobsters responded to glycine applied at 5×10^{-8} *M* by increasing flicking over only a 20–30 s interval, beginning about 10 s after the initial introduction of the stimulus solution. The onset of detection coincided with the timing of peak fluorescence as measured in trials in which dye was released. Therefore, the detection of glycine at low concentrations of this stimulus appears to be restricted to the period during which the level of introduced stimulus peaks.

Interestingly, 38% of all lobsters tested increased their antennular flicking in response to seawater (control); *i.e.*, in the absence of an applied glycine stimulus (Table III). Possibly, the lobsters were detecting substances introduced as contaminants during solution preparation, or issuing from other unspecified sources. Alternatively, antennule flicking may have been triggered by small changes in water flow, or by air bubbles produced when a valve was opened and closed during the introduction of solutions. To distinguish these possibilities, 20 trials were performed in which the valve was opened and then closed without stimulus or seawater being introduced. Six of the 20 lobsters tested responded with an increased rate of antennular flicking. The proportion responding (30%) is nearly identical to that when the seawater (control) was applied. Consequently, increased flicking in response to the seawater (control) is probably caused by the valve being opened and closed, not by the lobsters detecting some unidentified, stimulatory compound.

Discussion

To my knowledge, this is the first measurement ever of a threshold of "just noticeable difference" for chemoreception in an aquatic animal. The threshold concentration for glycine stimulus detection by the spiny lobster, *Panulirus interruptus*, is about 2–8% higher than the glycine background in seawater. Repeated measurements were made of the ratiometric dilution associated with the delivery of stimulus solutions to the experimental chambers, of the glycine concentrations in the stimulus solutions, and of the glycine concentrations in the seawater of the experimental chambers. The threshold determination was found to be very robust, changing little as a consequence of variation in stimulus dilution and glycine levels.

The threshold of *jnd* reported here for lobsters (2–8%) is slightly lower than those (4–33%) found for olfactory and gustatory detection by terrestrial animals, including humans (McBurney *et al.*, 1967; Shumake *et al.*, 1969; Pfaffman *et al.*, 1971; Cain, 1977a, b; Slotnick and Ptak, 1977). Direct chemical measurements of applied stimulus and background were made in only one of these studies (Cain, 1977a), and background concentrations fluctuated significantly. Because the applied stimulus was not always maintained above the mean background concentration, signal detection theory was used in estimating the likelihood of a given difference between stimulus and background (Green and Swets, 1974; Egan, 1975). Signal detection theory was not required in this study of lobsters because the applied stimulus was assumed always to be above background. The application of signal detection theory neither increases nor decreases estimates of *jnd* thresholds, but explains how variation in background stimuli influences threshold estimates.

An emerging theme in chemoreception research concerns mechanisms by which animals distinguish a stimulus from the background noise. Carr (1988) has hypothesized that compounds containing novel structural moieties provide the best chemical cues to marine animals. This hypothesis is appealing because it is advantageous for animals to detect signals that maximize the contrast between stimulus and background. Alternatively, substances occurring as DOM also serve as feeding cues to marine animals (Laverack, 1988). In such instances, the compounds providing the best cues may be those maintained at the lowest levels in seawater. Because glycine is abundant in seawater, yet is an effective stimulus detected by *P. interruptus* at concentrations only slightly above background, the results reported here suggest another alternative. It may be beneficial for animals to maximize differential sensitivity for signal molecules that otherwise lack unique structural moieties, but are strongly correlated with a valuable or limiting resource. Glycine, for example, is consistently one of three or four most abundant free amino acids in marine invertebrate flesh (Carr, 1988), occurring at 30–200 m*M*. Glycine, therefore, clearly signals the presence of potential prey, because marine invertebrates are the principal food resource exploited by lobsters (Lindberg, 1955).

The mechanism by which lobsters detect small differences between glycine stimulus and background is unknown, although sensory adaptation may be involved.

Adaptation is a change in sensitivity to an applied stimulus resulting from continuous prior exposure to a background stimulus. Stimulus backgrounds maintained for 2–3 min, or longer, are enough to influence the physiological response thresholds of chemoreceptors on the antennules of the Florida spiny lobster, *Panulirus argus* (Trapido-Rosenthal *et al.*, 1990), and on the walking legs of the American lobster, *Homarus americanus* (Borroni and Atema, 1988). In both cases, the effect is to mediate a parallel shift in dose-response functions corresponding to changes in the concentration of the adapting background. Insight may be gained by considering conclusions drawn from investigations on vision and hearing. Adaptation is thought to maintain the auditory and visual senses in working states that allow the detection of small, instantaneous changes in stimulus intensity; *i.e.*, <1% above background (*sensu* Keidel *et al.*, 1961). In this way, adaptation may act as an early filter, processing information and tuning animal responses to small differences between applied and background stimuli. Additional investigation will clearly be needed to more fully explore the interactions between sensory adaptation and chemical detection by *Panulirus interruptus*.

There is also an urgent need for future investigators of marine chemoreceptive behavior to measure background stimuli in seawater. Numerous studies have assayed responses by marine animals, representing nearly every major phylum, to a broad suite of stimuli, including amino acids, organic acids, sugars and nucleotides that also occur as DOM (see reviews of Linstedt, 1971; Carr, 1988; Laverack, 1988). In several previous studies, the relationship between applied dose and animal response has been examined, yet in none of them were the background levels of stimulant in the seawater of the experimental chambers measured. If the concentrations of stimulus in seawater had varied significantly between studies, then the differences in threshold response measured in these investigations may have been more apparent than real. Given that marine organisms live in a noisy chemical environment, measurements of the ability to distinguish between stimulus and background are required. Determinations of *jnd* thresholds, therefore, are critical to an understanding of chemically mediated behavior in the sea.

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